

### Incorporating Microbial Processes into Climate Change Models by Ann Reid

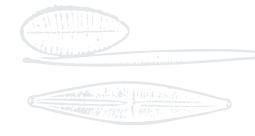
This report is based on a colloquium convened by the American Academy of Microbiology, February 21-23, 2011, in Dallas, TX.

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American Academy of Microbiology 1752 N Street, NW Washington, DC 20036

www.asm.org

#### **Participant list:**

#### **Co-Chairs**

Edward F. DeLong, Ph.D. Caroline S. Harwood, Ph.D.

#### **Steering Committee**

Penny W. Chisholm, Ph.D. David M. Karl, Ph.D. Mary Ann Moran, Ph.D. Thomas M. Schmidt, Ph.D. James M. Tiedje, Ph.D. Kathleen K. Treseder, Ph.D. Alexandra Z.

# American Academy of Microbiology Staff Ann Reid

Worden, Ph.D.

Sponsor Representative Joseph R. Graber, Ph.D.

#### **Participants**

Andrew Allen, Ph.D. Steve Allison, Ph.D. Ginger Armbrust, Ph.D. Antje Boetius, Ph.D. Zoe G. Cardon, Ph.D. Raffaele Ferrari, Ph.D. Mary K. Firestone, Ph.D. Michael (Mick) Follows, Ph.D. Rachel A. Foster, Ph.D. Beth A. Holland, Ph.D. Bruce A. Hungate, Ph.D. Samantha (Mandy) B. Joye, Ph.D. Gary M. King, Ph.D. Amala Mahadevan, Ph.D. Ann Pearson, Ph.D. William Reeburgh, Ph.D. Joshua Schimel, Ph.D. Rafel Simo, Ph.D. Robert L. Sinsabaugh, Ph.D. Bess B. Ward, Ph.D. Patricia L. Yager, Ph.D. Donald R. Zak. Ph.D. Jonathan P. Zehr. Ph.D.

### **EXECUTIVE SUMMARY**

#### **Odd Bedfellows**

It is difficult to imagine two fields more different in their methods, tools, and objectives than climate science and microbiology, and yet there is a vital connection between these two endeavors.

Microbes are critical players in every geochemical cycle relevant to climate including carbon, nitrogen, sulfur, and others. The sum total of microbial activity is enormous, but the **net** effect of microbial activities on the concentration of carbon dioxide and other climate-relevant gases is currently not known. The past two decades have witnessed an explosion in our recognition of the diversity of the microbial world, as new technologies have made it possible to characterize microbial communities in ever greater detail. Modeling, too, has experienced tremendous advances in its capabilities. For all the progress, however, we are not able to measure microbial processes in such a way as to allow climate scientists to include them in models of global climate.

Determining how to measure the rates, fates, and fluxes of climate-relevant gases through microbial communities and the environment is a task that falls between climate science and microbiology and is the focus of neither. While the gap between the two disciplines is daunting, the need to bridge it is urgent and the science and technology needed to begin to do so is within reach. By breaking the task down into tractable parts, strategically developing needed tools, methods and community resources, and facilitating the establishment of interdisciplinary teams with well-defined, shared goals, the task of incorporating microbial processes into climate models can begin to be tackled, to the benefit of both fields.

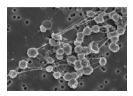
#### **Common Ground:**

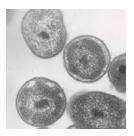
The differences between climate science and microbiology are considerable, but they have something quite powerful in common, the use of models. Indeed, both Earth's climate and microbial community processes are too complex to study without models. In both fields, models represent logical syntheses of assumptions and boundary conditions, can identify gaps in understanding, and are useful for revealing amplification and dampening effects. The development of methods to quantify

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microbial impacts on climate so that they can be incorporated into climate models is a major interdisciplinary challenge.

#### Connecting the pieces:

For the first time, an accurate, quantitative census of microbes inhabiting any environment can now be taken. Knowing which microbes are present is useful for suggesting the community potential, what processes might be going on now, or might be possible in other circumstances, but that information cannot yet be turned into fluxes and rates. The challenge will be to simplify complex community dynamics so that net inputs and outputs that accurately reflect reality can be incorporated into climate models.

The group recommended a multi-pronged approach to breaking the challenge into manageable parts.

#### **Recommendations:**

#### Choose a few specific biogeochemical cycles to serve as demonstration projects

Because the task is currently unmanageable in its entirety, the best approach would be to begin with in-depth characterization of particular biogeochemical transformations that are important, microbially driven, and tractable. Three examples meeting these criteria were identified at the colloquium.

#### Methane:

Microbes play important roles on both sides of the methane cycle—they both produce and consume methane. Human activities like cultivation and deforestation have substantial effects on methane fluxes. Furthermore, methane production from thawing permafrost in the Arctic could be large enough to be significant on a global level.

#### Carbon storage:

Microbes produce and break down an infinite variety of carbon compounds. Their activities are a primary determinant of whether any terrestrial or marine environment acts as a net carbon source or sink.

#### Nitrous oxide:

Nitrous oxide ( $N_2$ O) has more than 300 times the heat-trapping ability of carbon dioxide and human practices like agricultural fertilization have contributed to significant increases in nitrous oxide emissions over the last century. The actual rate of  $N_2$ O flux from agricultural soils varies enormously, due in large part to the activities of soil microbial communities.

#### Assess current data collection and develop a monitoring/data collection strategy

A great deal of pertinent data has been and continues to be collected that could be useful in efforts to understand microbial roles in geochemical processes. Unfortunately, these data are not organized in such a way as to make them accessible across the scientific community. Opportunities to leverage current activities are being lost.

### Implement validation processes to integrate data collection, modeling and experimentation

Bottom-up experimentation will have to scale from individual microbial physiologies in laboratory culture through to small-scale environmentally realistic mixed cultures. Top-down observation will require measuring relevant rates and fluxes while treating microbial community dynamics as a black box. Both will be needed to validate the inclusion of microbial processes in climate models.

#### Facilitate and provide incentives for collaborations and interdisciplinary training

Progress will require close interaction among communities that do not work together currently, and stable, long-term funding will be needed to lower the risk of participation. Interdisciplinary short-term training and long-term degree programs are key.

#### Address Technology Needs

Technologies like real-time remote sensing and improvements in pure and mixed laboratory culture techniques are needed to make it possible to collect data more inexpensively, more often, in more places, and to study microbes and microbial communities in new ways.

Major events of the distant past illustrate the need to incorporate microbial activities into existing climate models. They demonstrate that the microbial processes that affect climate do not necessarily balance each other out. Billions of years ago, changing microbial community composition resulted in the shift to an oxygenated atmosphere. The organisms that had inhabited the Earth for at least a billion years were no longer able to survive on the Earth's surface. In the past, such profound change took millions of years, a time span well beyond that with which current climate models are concerned. Today, changes due to human activity are causing similar large scale global effects in as little as 100 years. There is clear evidence that microbes can have an enormous impact on climate but their responses and impacts cannot currently be measured. In light of ongoing global change and the centrality of microbes in global biogeochemical cycles, their specific responses and activities in the context of climate change modeling can no longer be ignored.

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Billions of years

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# INCORPORATING MICROBIAL ACTIVITIES INTO CLIMATE MODELS

# Part I. What do microbes have to do with climate?

The living world plays a critical role in Earth's climate. One has only to imagine stepping from an asphalt parking lot onto a lush lawn on a hot summer day to recognize that life can change its surrounding physical conditions. But it isn't only grass and trees that affect climate, although they do, and powerfully. In fact, the most powerful impact of life on the Earth's climate is made by its smallest inhabitants — the microbes; Bacteria, Archaea, algae, fungi and other microbes may be too small to see, but are far too important to ignore.

The impact of microbes on climate goes back billions of years. It was microbes that first evolved the genetic machinery for nitrogen fixation — the capability to turn atmospheric nitrogen into a form usable by living organisms, and photosynthesis — the capability to use sunlight and carbon dioxide to make sugars and other complex molecules, with oxygen as a by-product. Over time, the oxygen from those trillions upon trillions of individual microbes built up in the atmosphere, eventually making the open air uninhabitable for Earth's earliest life forms (to which oxygen was toxic), and paving the way for the stupendous diversity of oxygen-breathing organisms that now inhabit our planet. Until the development of industrial processes to fix nitrogen just a century ago, and with the exception of a limited amount of atmospheric nitrogen that is fixed by lightning, all of life was dependent on microbes to convert nitrogen into usable form. As the earliest organisms evolved into more and more different life forms, complex food webs emerged and living organisms became critical players in the cycles of many elements: not only carbon, oxygen, and nitrogen, but also phosphorus, sulfur, iron, and many others.

Billions of years later, the Earth is still home to vast numbers of microbes. Each gram of soil contains, on average, 10<sup>8</sup> microorganisms. Each milliliter of seawater contains 10<sup>6</sup>. Even each individual plant and animal is home to a universe of microbes: as humans, we depend on the more than 10<sup>11</sup> microbes that live on our skin and in our guts to protect us from pathogens and help digest our food. Plants could not survive without the billions of microbes that live around their roots, providing them with essential nutrients. Our bodies contain 10 times more microbial than human cells.

And the same is true for nearly every other animal. In total, the Earth is home to an estimated 10<sup>30</sup> microbes — about half of Earth's biomass (Whitman *et al.*, 1998).

What does all this have to do with climate? The answer is a lot, because all of those microbes are still affecting the climate, just as they did billions of years ago. The sum total of their activity is enormous. But of course not all microbes are the same — some of them are producing oxygen, others are consuming it. Some are taking carbon dioxide out of the air, others are adding it. What is the net effect of all those activities on the concentration of carbon dioxide in the atmosphere? Is it possible that microbial activity will absorb all the extra carbon dioxide humans are adding to the atmosphere? Some of it? How much? Or will the rising global temperature affect microbial populations in such a way that they produce even more carbon dioxide?

The past two decades have witnessed an explosion in our recognition of the complexity of the microbial world. There are literally millions of different kinds of microbes living in complex, interdependent communities in every imaginable habitat. How the sum total of all of those interactions will affect global elemental cycles and climate is a critical, urgent, and fantastically difficult question to answer.

The situation is made more complicated by the reality that life is not static. When conditions change, living organisms evolve. So even if we knew everything about how microbes are affecting climate now, the changing climate will result in changed microbes, microbes that may affect the climate differently.





Major events of the distant past illustrate the need to incorporate microbial activities into existing climate models. We know that all of the microbial processes that affect climate do not necessarily balance each other out. Billions of years ago, changing microbial community composition resulted in the shift to an oxygenated atmosphere. The organisms that had inhabited the Earth for at least a billion years were no longer able to survive on the Earth's surface. In the past, such profound change took millions of years, a time span well beyond that with which current climate models are concerned. Changes due to human activity today are causing similar large scale global effects in as little as 100 years. There is clear evidence that microbes can have an enormous impact on climate but their responses and impacts are currently unmeasurable. In light of ongoing global change and the centrality of microbes in global biogeochemical cycles, their specific responses and activities in the context of climate change modeling can no longer be ignored.

Incorporating microbial processes into climate change models represents a great challenge. It is clear that microbes are important players in the Earth's climate and elemental cycles, but capturing all of their different activities, how those activities interact, and their net quantitative impact is daunting. There is good evidence that if climate models do not include microbial activities, they are likely to be missing critically important processes that over decades could dramatically affect how, and how quickly, climate changes.

# Part II: What are models and how do they work?

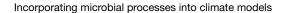
#### What do we mean by models?

When we talk about incorporating microbes into climate models, what exactly do we mean? Answering that question is easier if we define what we mean by models, and how climate models fit into modeling in general.

A model is a logical synthesis of assumptions and boundary conditions. Everyone uses models in this way every day, usually unconsciously — when we check the fuel gauge before driving onto the highway, we are mentally modeling whether we need to stop for fuel, factoring in distance and speed limits and our car's mileage (boundary conditions), how fast we plan to drive, whether there's likely to be a traffic jam, and many other variables (assumptions). Time and experience allow us to refine and improve these mental models. Similarly as new information becomes available to scientists, climate models are continuously revised to include more relevant variables and process rates.

Scientists and engineers use models to organize everything they know about a system into various pathways of cause and effect, depending on boundary conditions relevant to the system being modeled. Often the assumptions and boundary conditions can be described mathematically (e.g. every 10 mph increase in driving speed increases fuel consumption by 5%) and it is these mathematical models that are most relevant to this report's discussion of climate models. It should be kept in mind that models do not have to be quantitative, and conceptual models used by microbial ecologists are not. In many cases, microbial systems are not well-enough understood to be described by a system of equations; still, many of the pathways of cause and effect may be known and used to develop a conceptual model without specific quantitative relationships. These types of models are also informative-they allow us to explore the links and interactions between communities, develop hypotheses based on the model outputs, and test those hypotheses in nature. A model can be used to test hypotheses, and model outcomes can lead to new hypotheses and inform new experiments.

When one has organized all insights and assumptions about how a system works, and gathered information about the boundary conditions, a model can be used to test whether the assumptions are correct. This is especially helpful when the system being studied is complex; for example, you might hypothesize that properly inflated tires affect gas mileage, but the impact of that variable may be different depending on many other interacting variables such as external temperature, road conditions, or speed. Similarly, a climate model might be refined by incorporating the interaction of increasing temperature in the Arctic on microbial production of greenhouse gases. A model allows you to include all



One of the most valuable attributes of models is their ability to incorporate phenomena at many different scales. Like a Russian nesting doll, there can be models within models...

of the two-way interactions individually and provides information about how all those interactions collectively affect the system. It can also suggest that some interactions are missing or incorrectly characterized.

#### A model can identify gaps in understanding, and allows exploration of the importance of interactions and interdependencies.

It is important to test models against reality. "Validation" is the process of systematically checking the accuracy of the assumptions, boundary conditions and quantitative relationships used to build the model. When the model predicts a certain outcome, and the system is found to behave differently, there is reason to believe that something about the system has been misunderstood or left out. This characteristic makes models powerful learning tools, especially for complex systems with many feedback loops. Working back and forth between modeling and observation can be a highly effective way to identify areas where one's assumptions are faulty and point to questions that require further investigation. This "gap-finding" includes refining models to include known, relevant factors that were previously not accounted for. In the context of microbes and climate models for example, gap-finding might include incorporating the activities of methane-producing microbes into existing climate models.

#### A model need not be predictive to be useful.

It is understandable that we want climate models to predict exactly what the climate will be like in the future. But that is an unrealistic, even dangerous, expectation to have for models of a system as complex and incompletely understood as the Earth's climate. However, even if a model is not predictive, it is still useful in many ways — it can provide an expected range of outcomes, identify which variables are most sensitive to changing conditions, provide guidance on which interventions are likely to have the greatest impact, and identify areas where more research is needed. Models allow one to test assumptions against reality in an iterative fashion. As an example, it is known that oxygen depleted coastal ocean regions are currently expanding due to human input of nitrogenous wastes, which in turn stimulate microbial activity. It is not known however, how this increased activity will affect atmospheric CO<sub>2</sub> sequestration into ocean waters. Although the exact numbers cannot precisely be determined, the relative magnitude of the effect can be estimated by modeling, using a range of different assumptions.

#### Models can work across scales, and illuminate connections between scales.

One of the most valuable attributes of models is their ability to incorporate phenomena at many different scales. Like a Russian nesting doll, there can be models within models — small-scale phenomena (for example, rates of microbial respiration in forest soils) can be modeled and the net results fed into a larger-scale model (of, for example, net CO<sub>2</sub> metabolism in a forest). This ability to incorporate multiple scales is what eventually will allow climate models to take microbial processes into account. However, considerable effort will have to go into determining which small-scale microbial processes are most likely to have an important effect on large-scale outcomes. While this is currently beyond our ability, it will be important to figure out how to scale processes that occur at the level of individual microbes all the way up to net effects on climate-relevant variables.

Models are useful for revealing amplification and dampening effects.

The more complex a system, the more difficult it becomes to predict how a change in one component will ripple through the rest of the system. In many biological systems (the human body, for example), there are a series of feedback loops among components that serve to maintain homeostasis, keeping many system conditions within a particular range. Not all systems display this kind of self-regulating behavior — there can be systems that oscillate between very different conditions (predator-prey population sizes are a typical example), or systems with other dynamics that do not tend toward steady-state. Complex systems can show non-linear behaviors with time lags and thresholds (tipping points) and living systems, especially microbial ones with their impressive genetic flexibility, evolve over time. This is an important consideration with respect to microbes and climate change models; determining whether microbial processes will amplify the effects of climate change, dampen them, or have no net effect is a critical, and currently open question.

#### How do climate models fit into this picture?

The above general characteristics are true of all models; global and regional climate change models are no exception. A few characteristics of climate models stand out, however, that will make it especially challenging to incorporate the impact of microbes. First, global climate models are extraordinarily complex and computationally intensive. Second, changes in climate occur on a very long time scale — decades at the very least. Finally, experimentation — that is, intentional manipulation of variables to test whether modeling assumptions are correct — is essentially impossible at global and decadal scales. There is not a second Earth that scientists can use for experimental purposes, making modeling an important tool for us to understand how human activity will influence future climates,

The models used to understand how Earth's climate works include many different kinds of physical forces acting on scales from local (carbon emissions from individual cars) to global (the amount of sunlight striking the Earth). It takes a lot of force to cause a measurable shift in the climate. Small changes can eventually lead to detectable







climate impacts but it takes a long time for the signal from those small changes to emerge above the level of random variation. The smaller the signal, the longer it takes for the consequence to emerge from background. But that doesn't mean that small changes are irrelevant; if they persist, eventually their impact begins to be evident.

The consequences of these small pressures on climate may take a long time to make their presence felt, but they are inexorable. Like a big ocean liner that takes time and energy to turn around, once the consequences of these small changes on climate are recognized, reversing their effect will either take a very long time, or require very drastic changes.

The gradual increase in the atmosphere's concentration of carbon dioxide emissions due to human activities is an example of this kind of slow but inexorable change. Compared to the total amount of carbon dioxide in the atmosphere, and the natural annual fluxes through the biosphere, the amount added by human consumption of fossil fuel every year might seem trivial. But over time, as the concentration of that gas has gradually risen, the physical consequences are now clearly measurable.

Climate models include thousands of variables that interact in interesting and complicated ways — higher temperatures mean melting ice, ice reflects more sunlight than open water, so less ice means even higher temperatures — a positive feedback loop. On the other hand, higher temperatures mean more evaporation, which creates more clouds, which reflect more sunlight, creating a negative feedback loop. Atmospheric scientists, oceanographers, seismologists, geologists, physicists, and chemists are all involved in understanding these various physical forces and gathering the measurements needed to incorporate them into climate models. The models calculate the interactions of all of these different phenomena and provide estimations of how climate will change.

### Part III: Connecting the pieces

Satellite imaging of cloud cover and precipitation, submarine cables that monitor changes in temperature and salinity, sensors that can be deployed to provide many kinds of continuous, real-time data from remote locations — all of these technologies and more are providing ever more accurate and comprehensive physical data for climate models. Some of these technologies can also be applied to measuring biological phenomena. For example, satellites can monitor changes in chlorophyll levels and there exist both marine and terrestrial sensors capable of measuring concentrations of oxygen and carbon dioxide. Changes in chlorophyll and gas concentrations can be used as indirect measures of some climate-relevant activities of living organisms.

In addition to improvements in many physical measurement capabilities, our ability to characterize microbial populations in natural settings has exploded. For the first time, an accurate census of microbes inhabiting any environment can now be taken, using newly developed molecular biological methods. Adapting high-throughput sequencing technology spurred by the Human Genome Project has made it technically and economically feasible to sequence the collective DNA from whole microbial communities, rather than that of cultured isolates which represent only a tiny fraction of what is in nature. This approach, called metagenomics, has revealed a previously undreamed-of degree of diversity in the microbial world. Initially limited to DNA, microbial community analyses now embrace many "omics" approaches: transcriptomics detects RNA transcripts, giving a snapshot of what part of the community's genetic capability is being used; proteomics detects proteins; metabolomics detects the array of small molecules produced by the community. Together, the various 'omics, including rapidly improving techniques for single cell genomics, can provide a detailed picture of microbial community function, potential, and change over time, especially when taken in the context of other measurements of activity and growth of community members.

The challenge, however, is in somehow connecting this growing knowledge of "who is there?" and "what are they doing?" as reflected by "omics" data, to understanding how the communities work and measuring their net contribution to the fluxes of climate-relevant gases and aerosols. Complex microbial communities inhabit every imaginable environment, their composition changing over space, time, and environmental conditions. It would be ideal if a simple DNA test could determine microbial population structure, which could then be extrapolated to their net contribution to the fluxes of various climate-relevant elements. Unfortunately, this rosy scenario is at best distant and possibly unreachable. We have barely scratched the surface of the task of developing a "biogeography" for microbes. We know little about how microbial



communities vary across space and time. For example, for the purpose of measuring  $\mathrm{CO}_2$  flux, can we treat the ocean as one uniform microbial community acting uniformally over time? Or does community function and activity vary by region, season, or time of day? Or by temperature or available sunlight? Are the microbial communities in arctic soils essentially different from those in tropical soils? If so, how, and what are the implications for greenhouse gas emissions? These very basic questions have largely been unanswered, or answered only at the most general level.

A major challenge for microbial ecologists and climate modelers alike is to determine whether there are unifying principles, or identifiable features that can capture complex community dynamics so that some kind of net inputs and outputs can be incorporated into climate models. The goal will be to reduce the complexity to a manageable level while maintaining an accurate description of the system's traits. Connecting "omics" to processes and rates might be unachievable — the "omics" information is useful for telling us the community potential, what processes might be going on now, or might be possible in other circumstances, but that information cannot yet be turned into fluxes and rates. For that, we will need to know a great deal more about microbial physiology, evolution and ecology.

#### **Physiology**

Metagenomics data sets have now been collected in many environments, providing a microbial "parts list" of different microbial communities, but what all those genes do, or even which organism they come from, remain largely unanswered questions. Even for the bacterium E. coli and eukaryote yeast — undoubtedly the best characterized organisms on earth, we only know what 66% and 37% of their genes do (Karp et al., 2007). And that is what those genes do in pure culture under defined conditions. How all of those genes might work when the organisms are in a natural environment with thousands of other microbes is a black box of enormous proportions. In many cases, recreating important natural environmental factors in the laboratory — for example shifting light conditions in the upper ocean or physico-chemical gradients across soil microhabitats — is another challenge to characterizing microbial physiology. More detailed physiological understanding of various community members would help connect metagenomics data to overall community function, but physiological studies have traditionally been done on pure cultures in the laboratory. It will never be possible to culture every microbe, and even in those we can culture, we will not be able characterize every gene. Furthermore, all-important interactions cannot be addressed in pure culture. However, without detailed studies from the bottom up we will continue to flail against these great unknowns. Difficult though it is, the participants at the colloquium agreed that focusing on a limited number of model systems and characterizing some of the most important pathways in depth will be necessary to begin illuminating the connections between metagenomics and net rates and fluxes. This will require both advances in our ability to bring more microbes into pure culture (which has been challenging for many important environmental microbes), and rapid progress on the ability to maintain mixed cultures that model natural ones well enough to begin characterizing some of the inter-species feedbacks.



#### **Ecology**

There are too many different microbial assemblages in too many different environments to hope that even a small fraction of them could ever be brought into culture in the laboratory. Moreover, it is difficult to simulate the complexity of natural systems - both with respect to their varied inhabitants, and varying physical and chemical parameters — in the lab, once interactions are lost it can be difficult to know whether measured responses reflect how an organism might really behave in nature. Ecological studies can help bridge the gap between what is discovered in the lab and what is going on in the environment. Ecological approaches can uncover general rules governing community dynamics - including population fluxes, nutrient cycles, and food webs — and have the potential to uncover discontinuities, or tipping points, where a community's population structure shifts due to a change in conditions or oscillates because of some underlying interaction among species and their environment. Because ecologists routinely work at a theoretical level, informed by controlled experiments, iterative sampling, and modeling, their expertise provides a natural bridge between studies at the level of microbial physiology and the rates and fluxes needed by climate modelers.

Metagenomics can be used to bridge microbial physiology, ecology, and climate science, as it can allow scientists to diagnose and investigate characteristics, processes, and changes in ecosystems. For example, one study characterized vertically distributed, discreet microbial communities by sequencing the metagenomes of microbes at different depths. Community composition and architecture varied by strata reflecting changes in light availability and pressure. By identifying members of each individual stratum and their functional genes and metabolic properties a rough picture of each stratum's potential contribution to biogeochemical rates and fluxes emerges (DeLong et al., 2006). A similar study revealed the potential for metagenomics to diagnose evolutionary drivers in different ecosystems. Comparison of the metagenomes of specific strains of marine microbes revealed that in one location, genes for phosphate acquisition and metabolism were overrepresented, a sign that adaptation to phosphate scarcity is an important evolutionary driver in that location (Coleman and Chisholm, 2010). Such an approach could also be used to track changing conditions in a single ecosystem.

#### **Evolution**

A major challenge in the study of microbes, both in understanding their physiologies and their ecological dynamics, is their genetic plasticity. Many microbes can reproduce rapidly and sustain a relatively high mutation rate, providing many variants on which selection can act. Horizontal gene transfer among microbes expands this potential for variation. Finally, selection pressure from viruses can lead to rapid shifts in population structure. Taken in combination, microbial communities have a potential for rapid evolutionary change that adds an additional layer of complexity to the task of predicting how community function will change as environmental conditions shift. In a changing environment, microbes and microbial communities can very rapidly respond and adapt — and those adaptations have potential to feedback, either positively or negatively, on the direction and magnitude of environmental change.

By identifying members of each individual stratum and their functional genes and metabolic properties a rough picture of each stratum's potential contribution to biogeo-chemical rates and fluxes emerges.

#### Part IV: Action Plan

The participants in the colloquium who are most familiar with climate modeling efforts impressed upon the group that incorporating microbial processes into the global models is a challenge for the climate modeling community, especially as the underlying science seems to be advancing so rapidly. To microbial ecologists, the relevance of microbes to climate is clear, even if it is, admittedly, currently difficult to quantify. Thus, microbial ecologists must work with modelers to determine and then collect the needed quantitative information that would allow inclusion of these processes in models. The group recommended a multi-pronged approach to breaking the issue into manageable pieces.

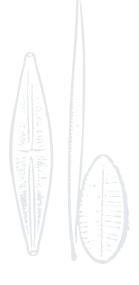
#### **Recommendations:**

- Focus on centrally important biogeochemical cycle demonstration projects.
- Identify immediate data collection priorities and begin collecting data as soon as possible.
- Facilitate an iterative validation process that includes data collection, modeling, and experimentation and involves researchers from many disciplines.
- Facilitate and provide incentives for multi-disciplinary training activities and collaborations that include microbial ecologists and climate modelers.
- Address technology and workforce needs.

# A. Focus on centrally important biogeochemical cycle demonstration projects.

The group suggested that because the task is currently unmanageable in its entirety, the best approach would be to begin with demonstration projects. In-depth characterization of particular microbial processes or specific biogeochemical transformation would require the development of technology and techniques that could later be transferred to other key biogeochemical processes. Evidence that these particular microbial processes do indeed make an immediate difference in the prediction of climate models would be a proof in principle that microbial processes must be taken into account. Several cycles were proposed. Participants aimed to identify "sweet spots" with the following characteristics:

Important: biogeochemical cycles or compounds (like greenhouse gases) that current models show to have a substantial effect on climate and to which models are sensitive



- Microbially driven: cycles that are particularly dependent on microbial processes, cycles in which living organisms make a big difference in magnitude or direction
- Tractable: cycles where the major players are known and tools are available to make rapid progress

The group at the colloquium identified three examples of cycles that fit these characteristics, but further community discussion is needed to refine or even revise these choices. It would be useful to include more climate modelers in that discussion, because their familiarity with the current models would allow them to provide feedback on variables to which the models appear to be especially sensitive, or where model behavior is suggesting gaps in knowledge.

#### Methane

Methane is a powerful greenhouse gas. According to the US Climate Science program (USCSP), methane traps heat in the atmosphere 20 times more effectively than carbon dioxide. Because it has a relatively short lifetime in the atmosphere of just 9-15 years, changes in methane levels have the potential to exacerbate or mitigate climate change relatively quickly. The IPCC estimates that approximately 60% of global methane emissions come from human activities. The other 40% come from natural sources. Interestingly, the official USCSP website does not mention microbes as a significant emission source, even though it is in fact microbes that are actually producing the methane in the wetlands, oceans, termites, and other natural sources cited there. Microbes are also the critical players in methane emissions from human-influenced activities like landfills, wastewater treatment, and agricultural sources like rice paddies and livestock.

Microbes play important roles on both sides of the methane cycle — they both produce (methanogenesis) and consume (methanotrophy) methane. Colloquium participants pointed out several ways in which understanding the role of microbial communities in the methane cycle is likely to have an important effect on climate models:

- Methanotrophs in soil are easily disturbed and slow to recover, so human activities like cultivation and deforestation may have long-term effects on methane fluxes.
- Methane production from thawing permafrost in the Arctic could be large enough to be significant on a global level; it is almost certainly likely to be relevant on a regional level.
- There could be amplifier effects between CO₂ and methane meta-analyses suggest that when CO₂ concentrations rise, so does methane production. (van Groenigen et al., 2011)
- Methane production is generally restricted to anaerobic environments (sediments for example) but it is also present in highly oxygenated waters including the surface ocean in concentrations that exceed the air saturated values. This suggests that there may be alternate pathways for methane production in the sea. It has been suggested that in some environments, plants transport methane directly to the atmosphere and there is little opportunity for microbial consumption. In other environments, microbes consume methane as it diffuses through soil and water around the plant. Regionally, methane

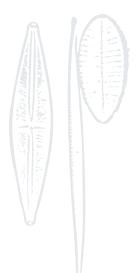
methane traps heat in the atmosphere 20 more effectively than carbon dioxide

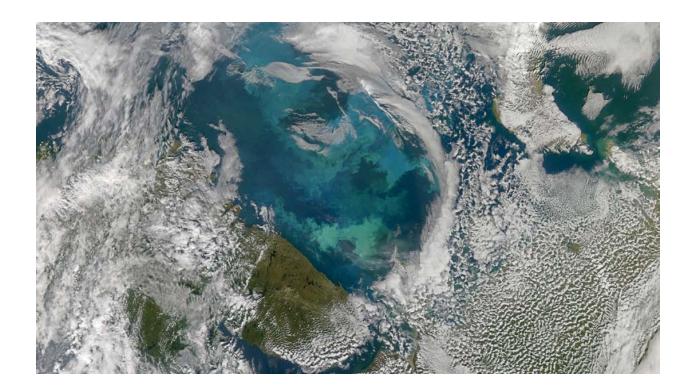
- consumption can vary between 15 and 80% of production, depending on which of these transport mechanisms is predominant. The bottom line is that there remain fundamental gaps in our understanding of the methane cycle.
- Technological and scientific advances are providing powerful new ways to study the methane cycle. The genes that are responsible for methanogenesis and methanotrophy are well-characterized, so that the capacity for both producing and consuming methane in a microbial community can be studied through metagenomic and gene array approaches. Techniques like SIP (Stable-Isotope-Probing) make it possible to monitor the progress of isotope-labeled methane through microbial communities and identify the key players.

#### Carbon

When we think of photosynthesis, we probably think first of terrestrial plants, but the capability to use solar energy to convert carbon dioxide into sugars and other energy-rich compounds first evolved in microbes. Microbes are still responsible for approximately half of the photosynthesis on Earth (Field et al., 1998). Because photosynthesis is the process by which carbon dioxide is removed from the atmosphere and converted into organic matter, it is a key process in the carbon cycle that must be included in climate models. Marine microbial communities are thought to be responsible for as much carbon fixation as land plants. Simple models of this phenomenon have marine microbes fixing carbon dioxide by photosynthesis and then either being eaten by larger organisms or sinking into the deep ocean reservoir, in the latter case, effectively sequestering the carbon more or less permanently. In reality, marine microbial communities interact through a more complex and dynamic set of interlocked processes and much of the carbon fixed by microbes in the upper layers of the ocean is not permanently sequestered, but rather is quickly released back into the water as waste products, or when the microbes are lysed by viruses. If the resultant dissolved organic carbon (DOC) is metabolized by other organisms living in surface waters, some of the carbon removed from the atmosphere by photosynthesis will quickly be released back into the atmosphere as CO<sub>2</sub>. Greater understanding of this pump will be needed if climate models are to incorporate biological carbon sequestration rates accurately.

The absorption of carbon dioxide by land plants also has a microbial dimension. Higher CO<sub>2</sub> levels lead to faster plant growth and consequent sequestration of atmospheric carbon in plant biomass. Plant growth, however, is limited by nitrogen and phosphate availability. Unless fertilizer is added, soil microbes must provide enough nutrients for plants to fully capitalize on higher CO<sub>2</sub> levels. Furthermore, whether a particular terrestrial ecosystem will be a net carbon source or sink depends on whether more carbon is converted into plant biomass than is released in the form of methane and carbon dioxide by soil microbes that feed on dead plant material or on the carbohydrates the plant supplies through its roots. Rates of decomposition, methanogenesis, and respiration, in turn, are also sensitive to other environmental parameters like temperature, soil moisture, and availability of nitrogen and phosphorus. CO<sub>2</sub> emission from soil, produced mostly by soil microorganisms, is about 10 times greater than the CO<sub>2</sub> that is globally produced by human fossil fuel combustion (Raich and Tufekciogul, 2000). Small changes in microbial respiration in soil have big implications for a global flux of CO<sub>2</sub> to the atmosphere. Achieving accurate estimates of net carbon fluxes will require determining the fate of various





carbon compounds in the complex and interconnected soil biome in a range of temperature and moisture conditions.

Two examples illustrate how simplistic assumptions about microbial impacts on the carbon cycle can be misleading or incomplete:

Phytoplankton blooms can be extensive enough to be observed by satellite, but it would be misleading to correlate chlorophyll concentration directly with photosynthetic activity as a way to estimate net primary productivity in the ocean. There is a backdrop of photosynthetic species that are omnipresent, rather than episodic bloomers and satellite measurements reflect only biomass at the surface. Colloquium participants pointed out, that depending on the species of phytoplankton responsible for the bloom, the consequences for carbon fluxes can be radically different. Small phytoplankton are more likely to be consumed by other organisms or killed by viruses, and in both cases, are thought to then release the carbon they fixed into the surface ocean food web where it is likely to be consumed by heterotrophs and consequently respired (and released into the atmosphere) as carbon dioxide or incorporated into the biomass of heterotrophic microbes in a process known as secondary production. Nevertheless, some recent studies have shown that small phytoplankton still contribute significantly to sequestration of carbon (Richardson and Jackson, 2007; Fawcett et al., 2011). Many climate models now include a dynamic representation of the phytoplankton community and do not rely only on satellite based estimates of production, but this remains an area of considerable uncertainty.

From 1994 to 1997, NASA conducted an extensive experiment to trace net fluxes of carbon dioxide in boreal forests. The experiment was motivated by the realization that atmospheric carbon dioxide levels were lower than expected based on the amount of CO<sub>2</sub> being released by human activities. Regional measurements suggested that the "missing" carbon appeared to be being absorbed north of latitude 40°N. The evergreen forests that carpet much of the far north represent a huge standing stock of sequestered carbon. The BOREAS measurements showed that carbon is absorbed in the summer when trees are growing, but much carbon is released in late summer when limited water supplies slows tree growth, yet warmed soils permit the active metabolism of soil microbes. During the four-year measurement period, the boreal forest's net annual carbon budget could be positive or negative, depending on the rate of microbial metabolism — a rate strongly influenced by water availability. Assuming that the ecosystem would be a carbon sink based solely on increased tree growth would therefore, not always be accurate.

Colloquium participants pointed out several ways in which understanding the role of microbial communities in carbon sequestration is likely to have an important effect on climate models:

- Who's there matters.
  - Phytoplankton blooms can have different consequences depending on which species is proliferating. Terrestrial plant growth and decomposition are dependent on the capacity of the associated microbial community to supply nutrients and break down dead material. Failure to understand the diversity in microbial communities could result in estimates of carbon sequestration rates that are too high or low, or even wrong in terms of whether the flux is positive or negative.
- The path from photosynthesis to long-term sequestration of carbon is considerably more complicated than previously appreciated.
  - The repertoire of microbial metabolic machinery for synthesizing and degrading carbon compounds is extremely diverse. The various genetic pathways for consumption, storage and degradation differ from microbe to microbe and are differentially activated depending on environmental conditions. There is an emerging awareness that the pools of particulate organic carbon (POC) and dissolved organic carbon (DOC) in the ocean and soil are heterogeneous and interconnected. DOC consists of many different compounds that vary in their susceptibility to degradation, requiring a division of that pool into at least two fractions labile and refractory.
- The role of phage in the marine microbial carbon pump is emerging as critical.
  - Knowing what compounds microbes are making and storing becomes even more important when the role of viruses in lysing microbes and releasing their contents into the ocean is taken into account.
- Just as understanding the role of phage is important, so is greater understanding of who preys on whom.
  - A consequence of discovering that microbial communities are far more diverse than previously suspected is the realization that microbial food webs are likely to be highly complex.

In both terrestrial and marine systems, technologies are maturing that will contribute to characterizing what kinds of carbon compounds are present in the environment, which microbes are producing them, which microbes are consuming them, and how those factors change as physical parameters like temperature, moisture, nutrients, or salinity change. It is conceivable that expression of particular genes associated with producing or consuming labile or recalcitrant carbon compounds may serve as biomarkers for rates of carbon sequestration. Like methane production and consumption, carbon sequestration is a process in which microbes are key, to which climate models are sensitive, and for which science and technology is poised to make rapid progress.

#### Nitrous oxide

Microbes play crucial roles throughout the global nitrogen cycle, which in turn has an important impact in climate. Developing a quantitative understanding of microbial contributions to every step of that cycle is a long-term grand challenge, but colloquium participants identified one component of the nitrogen cycle as especially important and potentially tractable. Nitrous oxide ( $N_2O$ ) is a component of the nitrogen cycle to which climate models are likely to be particularly sensitive because it has more than 300 times the heat-trapping ability of carbon dioxide. It also has a very long residence time in the atmosphere, so once in the atmosphere, it is removed only very slowly. It is produced as a byproduct of two important microbially-mediated steps in the nitrogen cycle. Under aerobic conditions, nitrifying bacteria release  $N_2O$  during the process of converting ammonium to nitrite. Under anoxic conditions, there are microbes that 'breathe' nitrate instead of oxygen, producing either  $N_2O$  or nitrogen gas in the process, depending on environmental conditions and which denitrifyers are present.

Colloquium participants pointed out several ways in which incorporating the microbial production of  $N_2O$  could significantly affect climate models:

- Human practices have contributed to significant increases in nitrous oxide emissions over the last century. Agricultural soil is a prime source of human-associated emissions contributing 68% of such N<sub>2</sub>O emissions in the United States in 2008. (EPA, 2011). Emission rates are tied to use of nitrogen fertilizers but are strongly affected by land management practices. Livestock manure is also a major source of N<sub>2</sub>O and, again, management practices have been developed that can reduce emission rates. Understanding the microbial contribution to N<sub>2</sub>O from these sources could contribute to making management practices even more effective. Currently, estimates and averages are used to model overall agricultural emissions of N<sub>2</sub>O, but direct measurements vary widely from field to field. Much of that variation is attributable to variations in microbial activity because of differences in temperature, moisture and other soil characteristics. Inclusion of microbial ecologists in modeling such emissions could improve their accuracy.
- An indirect agricultural influence on N<sub>2</sub>O emissions results from fertilizer run-off into streams, rivers and estuaries. Again, it is microbial communities that drive the conversion of these nitrogen sources to N<sub>2</sub>O, so understanding their dynamics could provide more accurate estimates for climate modelers.

Nitrous oxide (N<sub>2</sub>O) is a component of the nitrogen cycle to which climate models are likely to be particularly sensitive because it has more than 300 times the heat-trapping ability of carbon dioxide.





- The ocean is estimated to be responsible for approximately one-third of global N₂O emissions, a percentage that has been thought to be stable because of the relative immunity of the open ocean from anthropogenic influences. However, high inputs of human-associated nitrogenous wastes and runoffs and slow ventilation rates in coastal regions have stimulated microbial activities that generate oxygen minimum zones. These OMZs produce N₂O at levels hundreds of times above the open ocean average and OMZ's could increase in volume due to excess nitrogen inputs and increased stratification resulting from global warming. The increasing number and size of OMZs could have an effect on the overall marine N₂O budget. In any event, the open ocean is so massive that even minute changes in gas budgets in the ocean can have a global effect (Duce *et al.*, 2008).
- At the same time, some studies suggest that ocean acidification a decrease in the ocean's pH due to rising atmospheric CO<sub>2</sub> levels may reduce N<sub>2</sub>O emission rates through physiological effects on the microbes that produce it. Understanding the effect of changes in pH on ammonium oxidizers in the ocean, then, would contribute to better estimates of future N<sub>2</sub>O emissions.

The methane balance, carbon sequestration process and N<sub>2</sub>O production, of course, do not exist in isolation, either from each other or from other biogeochemical activities. Making progress on any or all of them will take a concerted effort but will certainly shed light on many important feedback loops affecting climate.

# B. Assess current data collection and develop a monitoring/data collection strategy.

Making progress on any of the above biogeochemical processes would require considerable data collection. But whether or not the three demonstration projects above are chosen for in-depth study, colloquium participants agreed that a general lack of data, and especially a dearth of data in the form amenable to incorporation into models, is one of the greatest roadblocks to incorporating microbial processes into climate models. Colloquium participants acknowledged that a great deal of pertinent data has been and continues to be collected. But there are (at least) two major challenges on the data collection front — the amount of data that could be collected is virtually infinite and long time series are extremely challenging logistically, but absolutely critical scientifically. So even though it is very difficult to foresee which data will be most useful in the long run, and the appropriate time and distance scales are as yet unclear, the group agreed that it is important to get started now. It will no doubt be necessary to undertake some new kinds of data collection but there was a shared sense that a great deal of data currently being collected could be useful in efforts to understand microbial roles in geochemical processes.

The group pointed out opportunities to leverage current activities for the purpose of data collection that are being lost currently.

- Climate modelers may be unaware of data being collected by microbial ecologists and vice versa, and data may be collected or stored in a way that limits their utility to the other group. A two-way dialogue is critical to help steer future observations in order to maximize the utility of each community's data products.
- Grants focused on the study of particular systems rarely include adequate support for general data collection. Adding rate and flux measurements to ongoing microbial ecology studies could provide an abundance of data that would be useful to climate modelers at a relatively modest marginal cost. However, it is unlikely that support would be forthcoming for data collection not directly needed for the proposed research project. Especially in the current funding climate, it is difficult for any agency to justify support for additional data gathering even if it would clearly further the mission of other agencies.

Because data are currently being collected for so many different purposes across many federal programs, achieving the above goals will require a joint effort involving several currently unconnected scientific communities and the agencies that fund them. The group recommends a series of focused workshops to convene scientists and program officers from across the relevant agencies (DOE, NOAA, NASA, NSF, USDA, EPA, and others) whose portfolios include efforts to collect and organize data relevant to each of the demonstration projects. Data need to flow in several directions — for example, data on changes in ocean circulation, stratification and mixing will be important to microbial ecologists, whose data on changing population structures and community function could contribute to more nuanced understanding of the climate impact of physical changes in ocean layers. Each workshop could consider the following:

 Survey what is already being collected and make sure it is being fully utilized. Ask whether any of it can be used to help determine how

...small-scale experiments may help to demonstrate aspects of microbial systems that are especially sensitive to environmental change or susceptible to tipping points characteristics that would justify the investment needed to scale up to inclusion in global models.

- frequently and where new measurements need to be taken to begin to account for variation in microbial community inputs and outputs.
- Collaborative efforts to improve the basic physiological and biochemical descriptions of microbial processes employed by modelers.
- Use models to help guide observational strategies in the field. Global-scale climate models bring together understanding of cellular scale physiology and biogeochemistry, and environmental parameters. Thus appropriate models could be used to identify or refine testable hypotheses regarding the relationship of microbial communities and gradients in the ocean or soil environments.
- Reassess standard measurements and assemble existing measurements into a form useful to modelers.
- If possible, identify and develop biomarkers that correlate with functional boundaries and that have the potential to serve as proxies for entire communities or processes.
- Determine what flux measurements can be taken now and begin gathering data.
- Build on time series programs that are already underway.
- Build climate-relevant data gathering into specific microbial ecology projects (e.g. general physical, chemical, and geographical information), and microbially-relevant data gathering into system-level data gathering efforts.

Charting a way for agencies to leverage each other's research investments is a daunting challenge, but failure to coordinate efforts means losing promising opportunities to collect data in a cost-effective manner. Clearly, it is not possible to collect everything, everywhere, continuously. Nevertheless, since long time series are crucial, and since we are rapidly losing the window of opportunity to collect baseline data in advance of climate change, it is important to set priorities and begin coordinating current data and collecting the new data that has been identified as highest priority as soon as possible.

# C. Implement a variety of validation processes involving researchers from many disciplines integrating data collection, modeling, and experimentation.

Validation is the process by which models are demonstrated to accurately reflect the behavior of the target system. The process of validation is an iterative one, with initial assumptions measured and tested at small scales, then scaled up and tested against large-scale datasets. Validation also includes sensitivity analyses that identify which variables have important effects on model behavior and therefore are most important to measure. In many cases, the process of testing involves experimentation or manipulation — for example, growing plants under elevated CO<sub>2</sub> conditions, or adding iron to the ocean and measuring changes in productivity. In the case of the microbial processes discussed at the colloquium, small-scale experiments may help to demonstrate aspects of microbial systems that are especially sensitive to environmental change or susceptible to tipping points — characteristics that would justify the investment needed to scale up to inclusion in global models. For many microbial processes, however, experimentation will be difficult and other means will be needed to validate models. Current climate models are validated against global measurements of CO<sub>2</sub> and oxygen levels, net productivity estimates based on remotely sensed chlorophyll distributions and terrestrial plant biomass, and time series of

global temperature and sea surface height measurements. Sometimes paleo-data derived from fossil or ice-core samples can be used to validate modern models.

In order to validate models that incorporate microbes, it will be necessary to proceed both from the bottom up and the top down. Bottom-up experimentation will have to scale from individual microbial physiologies and simple community interactions in laboratory culture to small-scale in situ studies. Top-down validation of the microbial components of models could include coordinated efforts to correlate changes in microbial communities through metagenomics, transcriptomics and proteomics with changes in physical parameters. Models are also validated from the "top down" using global-scale datasets such as time series of atmospheric CO<sub>2</sub> and methane concentrations.

The group identified a number of areas where investment is needed to facilitate working from the "bottom up":

- Development of improved techniques for culturing ecologically relevant model organisms that are currently non-represented — lack of representation is a problem across environments (both soil and marine) and across domains (bacterial, archaeal and eukaryotic)
- Advances in techniques for growing microbes in mixed culture and environmentally-realistic conditions
- Single cell technologies
- Laboratory evolution experiments
- High throughput gene function assays and experimental platforms

#### And from the "top down":

- Better and more extensive reference genomes
- More concerted efforts to map the biogeography of microorganisms
- Approaches to capturing episodic or non-linear events (for example, N<sub>2</sub>O emissions from oxygen minimum zones or from soils)
- In-situ measurements of growth and activity rates in the ocean and soil
- Tracer and isotopic approaches to link taxonomy and genes with actual rates
- New approaches for measuring turnover and mortality of marine plankton and soil microbes

If both top-down and bottom-up approaches are organized in a coordinated way around a particular biogeochemical process (like those suggested above), progress could be rapid. Intermediate experiments like mesocosms and attention to the development of computational genomics and functional modeling should be considered as a way to link the top-down and bottom-up approaches.





### D. Facilitate and provide incentives for collaborations and support interdisciplinary training activities.

Progress will require close interaction among communities that do not necessarily work together currently. For example, the EPA is tasked with developing estimates of methane and N<sub>2</sub>O emissions from natural and human-influenced environments. Both "top-down" (for example, estimating methane emissions by multiplying the number of acres of rice cultivated in the U.S. by the average methane emission per acre of rice) and "bottom-up" (measuring actual emissions from rice paddies to estimate average emission rates) approaches are used to develop average fluxes. Actual emission rates vary widely. The inclusion of microbial ecologists in these efforts would improve understanding of the source of the variation and allow the development of biomarkers that could improve estimates. Establishing such teams would set in motion a virtuous cycle of reconciling observational data and experimental results with model outcomes, with benefits all around — models that better reflect environmental conditions, new and testable scientific hypotheses, and better targeted data collection.

Participation in non-traditional collaborations across institutions and disciplines requires researchers to take a significant risk; funding mechanisms, publications, and academic reward structures are all organized along traditional disciplinary lines. Generally teams function best if they self-assemble, so efforts on the part of funding agencies to encourage groups of researchers to plan and propose collaborative efforts will be needed. Furthermore, funding will have to be substantial enough, both in amount and in duration, to lower the risk of participation.

The establishment of teams of researchers made up of individuals with different disciplinary expertise offers a promising opportunity for multi-disciplinary education and training. Participants at the colloquium expressed enthusiasm for programs that allow students to gain meaningful experience of more than one field. As it now stands, neither academia nor federal research agencies have institutional mechanisms to assemble a scientific team with the range of expertise needed to attack this challenge. An important part of moving forward is development of a research infrastructure capable of working from microbial gene expression to global climate. At present, there are no degree programs that span the range of expertise needed to assemble such a team — this could be seen as a national need. Whether this is done via exchanging graduate and post-doctoral students between laboratories, graduate programs that bridge fields, or summer courses that bring both students and faculty from different disciplines together for intensive collaborative exercises, the group emphasized that developing a cohort of students "fluent" in more than one discipline will be a critical factor in successfully tying microbial ecology to climate modeling.

#### E. Address technology needs.

#### Technology needs:

Technology development is needed for two broad purposes: first, we need technology that improves data collection by making it possible to collect data more inexpensively, more often or continuously, and in more places. The group emphasized technologies that are simple, cheap, and easy to distribute across networks.

Secondly, we need technologies that allow microbes and microbial communities to be studied in new ways. The exact mix of technologies needed will depend on the outcome of discussions about data collection priorities (recommendation B) and the particular demonstration projects chosen (recommendation A). As a place to start, the following ideas were identified during the colloquium:

#### Data collection technologies:

- Ability to carry out remote sensing of distribution of DOC, POC, and phytoplankton functional groups in the ocean surface layer
- Optics approaches for measurement of phytoplankton distribution, abundance and activity
- Ocean and soil in-situ sensors to measure methane concentrations
- Real-time sensors of in-situ extracellular enzyme activity
- Real-time micro sensors for soils, including biological sensors
- Network of porous samplers to probe ocean sediments
- Multi-functional microfluidics sensors for ocean waters and sediments
- Integrated measurement of key gas fluxes
- Switch markers that indicate which direction processes are occurring (e.g., production vs. consumption)



# Parameterizations of microbial physiology employed in the ocean component of climate models are still typically based on concepts from the 1940s ... or the 1960s ... yet very significant advances have occurred in the past several decades ...

#### Modeling technologies:

Parameterizations of microbial physiology employed in the ocean component of climate models are still typically based on concepts from the 1940s (Monod kinetics, fixed "Redfieldian" elemental ratios) or the 1960s (Droop/Caperon internal stores model) yet very significant advances have occurred in the past several decades in cell biology, methods of observing and characterizing microbial systems and modeling cell physiology. In particular there have been enormous advances in genomic, molecular and metabolic understanding of cell physiology and omics and meta-omics methods have provided new ways to "observe" microbial communities. Furthermore, techniques to model regulatory and metabolic networks at the cellular level — systems biology approaches — have grown increasingly sophisticated. The following areas could be fruitful:

- Develop physiological parameterizations which directly interface with omics and meta-omics data and perspectives, which may require radical paradigm changes in modeling tools and approaches
- Learn from and potentially adopt, physiological models and tools currently employed in genome-scale modeling of microbes and cellular scale systems biology (for example, flux balance analysis)

#### Analytical technologies and community resources:

- Ability to culture model organisms from currently uncultured groups, and ability to culture model microbial communities
- Full genome sequences of many more microbes to serve as scaffolds for metagenomic data
- Ability to measure carbon use efficiency (growth efficiency) of microbes under different temperatures
- Improvements in long read single molecule sequencing
- Improvements in assembly and interpretation of "omics" data
- Ability to conduct experiments, measure rates of processes and monitor shifts in community composition under in-situ conditions
- Improvements in micro-scale techniques like NanoSIMS, SIP for interrogating single cells and microenvironments
- Better techniques for transcriptomics and proteomics in soils
- Improvement in spectrometry to allow measurement of microbial community structure
- Ability to determine chemical composition of DOM in ocean and freshwater systems and soil organic matter in terrestrial systems
- Ability to determine rate and mechanism of POC to DOC conversion and to determine the fraction of refractory versus labile DOC

#### Part V: Conclusion

Bridging the gap between climate science and microbiology is a daunting task, but the need to do so is urgent and the necessary science and technologies are within reach. By breaking the task down into tractable parts, strategically developing needed tools, methods and community resources, facilitating the establishment of interdisciplinary teams with well-defined, shared goals, and training a new generation of researchers to be fluent in both fields, the goal of incorporating microbial processes into climate models can begin to be tackled, to the benefit of both fields. With carefully targeted efforts, incorporating microbial activities into global climate models should not remain out of reach forever.



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